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Sedegah M, Leef MF, Matheny S, Beaudoin RL

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MKD group, there were statistically significant ($P < 0.05$) increases in mean prothrombin time (8.9 vs. 7.4 sec), alkaline phosphatase content (184 vs. 115 units), total bilirubin (3.9 vs. 0.4 mg %), and triglycerides (557 vs. 100 mg %), and statistically significant ($P < 0.05$) decreases in mean total protein (3.5 vs. 4.6 mg %) and cholesterol (28 vs. 94 mg %), but all these experimental parameters normalized by day 12 in surviving hamsters. Pathologic examination on day 5 revealed pale livers in all of the hamsters in the 26-, 104-, and 416-MKD groups, and pale or dark kidneys in 2/5 and 5/5 of the 104-MKD and 416-MKD groups, respectively. In addition, the 104-MKD and 416-MKD hamsters that died prematurely generally had reddened (apparently hemorrhagic) areas in the stomach and cecum. Histopathologic investigation revealed necrosis of individual hepatocytes in the 416-MKD group, and a trend to hepatic vacuolization and vacuolization of the kidney tubule epithelium with increasing FB dosages.

In brief, the hamsters that received the approximate LD_{50} (104 MKD of FB for 4 days) experienced reversible elevations in liver function tests, discoloration and cellular vacuolization in the liver and kidney, and (in moribund animals) hemorrhagic areas in the gastrointestinal tract.

For dog experiments, 6-mo male beagles weighing approximately 10 kg were inoculated intravenously with 1×10^8 *L. donovani* amastigotes (Chapman et al., 1979, Revista do Instituto de Medicina Tropical de São Paulo 21: 189–193). After 13 days, the dogs (in groups of 2 or 3) were administered FB in gelatin capsules per os at dosages of 0, 3.25, 6.5, 13, or 20 MKD (divided BID) each day for 4 days. The spleens and livers of dogs administered 3.25 or 6.5 MKD had 52% of the parasite burden of control dogs 1 day after the last day of therapy. All dogs given 13 or 20 MKD were moribund on days 3–4 of therapy. Clinical signs were vomiting, loss of appetite, listlessness, loss of weight, dehydration, and bloody diarrhea or frank blood per rectum. Lesions seen at necropsy of these dogs were yellow, fatty livers and frank blood in the intestines. Comparison of data from the sera from these animals to that from the other 3 groups of dogs revealed significant ($P < 0.05$) increases in the mean blood urea nitrogen (50 vs. 22 mg %), alanine aminotransferase (48 vs. 20 mg %), and uric acid (1.8 vs. 0.77 mg %), and significant ($P < 0.05$) decreases in the sodium (131 vs. 144 mg/L) and chloride (93 vs. 114 mg/L). Apparently, the lethal mechanism of oral FB for beagles involves liver and kidney damage, hemorrhagic enteritis, and hyponatremic dehydration.

The activity and toxicity data of this and the prior study indicate that formycin B administered orally to hamsters on days 1–4 had a modest therapeutic index when determined on day 5: the approximate ED_{92} = 13 MKD, ED_{98} = 52 MKD, and LD_{50} = 104 MKD. In beagles a favorable therapeutic index was not achieved. In both species of animals examined on day 5 and in hamsters examined on day 12, marked changes in formed blood elements were not generally seen. Rather, toxicity particularly affected the liver, the kidney, and the gastrointestinal tract, and animals appeared to die from gastrointestinal hemorrhage.

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A Rapid Method for Screening Antibodies to *Plasmodium yoelii* Liver Stages by Immunofluorescence

Martha Sedegah*, Mary F. Leef*, Steven Mathenyt, and Richard L. Beaudoin*, *Malaria Division, Infectious Diseases Department, Naval Medical Research Institute, Bethesda, Maryland 20814, and †Biomedical Research Institute, 12111 Parklawn Drive, Rockville, Maryland 20852

The developmental stages of plasmodia receiving the most attention in the search for a malaria vaccine are sporozoites, blood stages, and gametes. The liver stages have been the least studied. Nonetheless, recent evidence has shown that liver stages (LS) of *Plasmodium falciparum* have their own unique stage-specific antigens (Druilhe et al., 1984, American Journal of Trop-

ical Medicine and Hygiene 33: 336–341), and conceivably the liver stages may eventually provide antigens with demonstrated capability of inducing protective immunity at the level of the liver. Such candidates may be useful in fortifying the prophylactic protection obtained from vaccines based on sporozoites alone. In addition, the presence of circumsporozoite (CS) antigen in trophozoites that develop from irradiated and nonirradiated sporozoites implicates the liver stages in the development of malarial immunity (Sigler et al., 1984, American Journal of Tropical Medicine and Hygiene 33: 544–547; Mazier et al., 1986, Science 231: 156–159).

The indirect fluorescent antibody test (IFAT) has been applied in assessing immune responses to malaria infections and in developing hybridomas secreting antibodies directed against stage-specific antigens. The IFA technique applied to LS relies on the use of either thin frozen liver sections or sections cut from blocks of liver fixed in Carnoy's solution as the antigen. The use of such preparations in an IFAT for screening large numbers of hybridoma supernatants or for testing large numbers of different immune sera is both time-consuming and tedious, especially when the sera have to be titered individually. The present report describes a rapid, simple method of preparing high-quality antigen slides of LS of *Plasmodium yoelii*. The method can also be applied to any rodent malaria parasite.

Thorax sporozoites of *P. yoelii* 17 X (NL) strain, maintained by cyclic passage through *Anopheles stephensi* and mice, were harvested by discontinuous gradient centrifugation 14 days after the infection of 3-day-old mosquitoes (Pacheco et al., 1979, Journal of Parasitology 65: 414–417). Using varying doses of injected sporozoites, it was found that a minimum inoculum of 3×10^6 sporozoites injected intravenously (i.v.) into a 6- to 8-wk-old Balb/CBYJ mouse (Jackson Laboratories) routinely resulted in satisfactory preparations. Forty-four hours after inoculation the whole liver was removed. A piece of the liver was fixed in Carnoy's solution, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. A total of 100 microscope fields were examined with a low-power (10×) objective for liver stages. The mean number of LS per 10 fields was 19.5 ± 2.1 in 1 experiment when 7.7×10^6 sporozoites were injected i.v.

The remaining infected liver was used to prepare a suspension of hepatocytes in Medium 199, containing 5% normal mouse serum. The liver was minced and then pressed through a stainless steel sieve (0.012-inch mesh) sitting in a petri dish with the rubber-tipped plunger of a 10-ml syringe. The cell suspension was collected into a 15-ml tube and placed on ice. After large pieces had been allowed to settle by gravity, supernatant containing suspended liver cells was collected into a second tube. The cells were washed and concentrated by centrifugation for 10 min at 500 *g* in a refrigerated centrifuge set at 4 C. The total number of cells was estimated after counting a sample in a hemocytometer. The hepatocyte suspension was used to make antigen slides for IFA studies. It was important to prepare very thin smears in the antigen slide wells because liver stages were difficult to recognize unless only a single layer of cells was present; furthermore, thick smears tended to wash off during IFAT procedures. Good antigen preparations were obtained by placing 20–25 × 10^3 cells from an infected liver suspension into each well of an IFAT antigen slide. This could be achieved by spreading 1 μl of a 20–25 × 10^6 cells/ml suspension in each well with a pipette tip. This method made it possible to count the total number of liver-stage parasites in a given concentration of liver cells. The method can be further simplified by using a pasteur pipette to draw up approximately 0.2-ml aliquots of the liver suspension. After placing

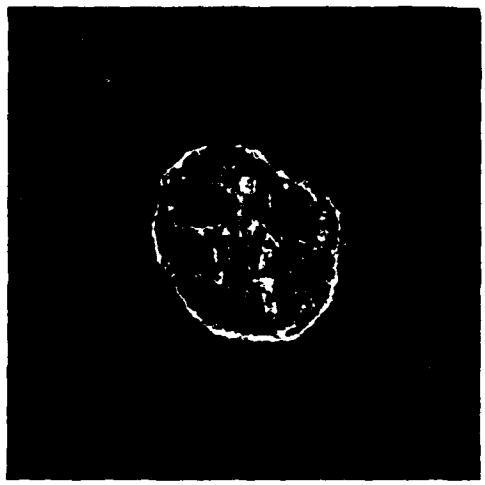

FIGURE 1. Immunofluorescent staining of *P. yoelii* liver stages by antisera from mice immunized with 3 doses of 6×10^4 infective sporozoites maintained on chloroquine. Dried liver-stage antigen was prepared from infected liver 44 hr after intravenous injection of a Balb C/BYJ mouse with 7.7×10^6 sporozoites (×400).

enough of the suspension to cover the first well, the suspension is drawn back into the pipette, leaving a very thin film in the well. The procedure is repeated for the next well, and so on. This modification provides a very quick method of preparing large numbers of antigen slides and was also noted to be delivering approximately 1 μl/well. With either method, the suspension is allowed to dry and can be stored with desiccant at −70 C until needed. Preparations stored in this way have now remained stable for up to 9 mo.

Immune serum reacting with liver forms was prepared from mice infected 3 times with a large inoculum (6×10^4) of sporozoites at weekly intervals. Mice were maintained on chloroquine (10 mg/kg body weight) given every other day to limit parasitemia (Beaudoin et al., 1977, Experimental Parasitology 42: 1–5). This procedure produces substantial anti-liver-stage as well as anti-sporozoite antibody. A standard IFAT protocol was then followed.

The frozen liver-stage antigen preparations were thawed and dried in a glass desiccator. Test serum diluted in filtered PBS, pH 7.4, was applied to the well containing the antigen. After the slides were incubated at 37 C for 30 min, they were washed in filtered PBS for 20 min and incubated again with FITC-labeled rabbit anti-mouse IgG (Miles Laboratory, Naperville, IL) for 30 min at 37 C. After a second washing, slides were mounted in buffered glycerol and examined under a fluorescent microscope. A low power objective (10×) was first used to scan each well to count the number of liver stages present, then these were confirmed using higher magnification (40× objective) (Fig. 1).

Although the technique was devised for the study of the liver stages of malaria parasites, it obviously could be applied to preparations of other parasites of the liver as well as other organs such as the spleen.

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The Role of Rat C3 and C3 Receptor-Bearing Alveolar Macrophages in *In Vitro* Attrition of Infective Larvae of *Nippostrongylus brasiliensis*

Thomas G. Egwang, Naval Biosciences Laboratory, Building 844, Naval Supply Center, Oakland, California 94625; **Jack Gauldie,** Host Resistance Program, Department of Pathology, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5; **and A. Dean Befus*,** University of Calgary, Health Sciences Center, 3330 Hospital Drive, NW, Calgary, Alberta, Canada T2N 4N1. *Corresponding author

In outbred, Sprague-Dawley rats infected with the nematode *Nippostrongylus brasiliensis*, C3 has been implicated in the mechanism of *in vitro* killing of infective larvae (L3) by alveolar macrophages (Egwang et al., 1984, Clinical and Experimental Immunology 55: 149–156). First, in the presence of normal rat serum, rat C3 could be detected on the surface of parasites. Second, killing occurred only under conditions in which C3 was present in the medium and deposited on the surface of L3. Lastly, the recognition of C3-coated L3 required effector cells bearing C3 receptors. Thus the extent of helminthocidal activity of broncho-alveolar leukocytes correlated with their content of C3 receptor-bearing alveolar macrophages which increased during infection. Herein we present further evidence for the possible role of C3 and C3 receptors in lung re-